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The effect of using gelatin membrane loaded with copper oxide nanoparticles and selenium on some properties of soft cheese

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A B S T R A C T

This study was conducted in the laboratories of the Department of Food Sciences - College of Agriculture and Forestry, University of Mosul ,during the period 20/2/2022 to 5/2/2023. Soft cheese was made from cow's milk covered with gelatin membranes which is loaded with copper oxide and selenium nanoparticles. The specimen stored for 20 days at a temperature of 5±2°C. The chemical properties of cheese were studied in terms of moisture, fat, protein, ash, and pH. The results showed that the moisture content decreased during the storage period The treatments coated with gelatin films to which selenium particles and copper oxide nanoparticles were added, had the lowest moisture loss percentage, and the T8 treatment had the lowest moisture loss percentage. The rest of the cheese components of protein, fat and ash did not show significant differences on the first day of storage compared to the two comparison samples without packaging and covered with gelatin film only T1 and T2, but the percentages of these components increased at the end of the storage period in proportion to the loss of moisture. The pH values were between 5.9 and 6.3% for the treatments coated with gelatin films supported by copper oxide and selenium nanoparticles, compared to the two control samples, T1 and T2. The pH values were 5.4 and 6.3, respectively.

Introduction

Cheese industry is one of the main industries in the field of dairy that has been known to man for a long time. It witnessed a rapid development in the second half of the nineteenth century. There are more than 800 types of cheese sold in the world under different forms and names. In view of the cheese content of nutrients such as proteins, fats, mineral elements, and vitamins, in addition to its moisture content (50–70%) which makes it a suitable medium for the growth of various types of microorganisms including bacteria, yeasts, and molds. Therefore, its preservation period does not exceed several days (El-Shafei et al., 1992). In view of the importance of cheese as a food that is widely consumed and because it can be exposed to contamination with microorganisms that cause food poisoning during the stages of production, processing, marketing, or storage, directly or indirectly, soft cheese was chosen in this study.

There was a need to use biomaterials in food packaging to reduce the use of non-biodegradable and environmentally polluting plastic materials. On the other hand, biomaterials represented by natural plant or animal materials such as proteins, sugar, and fats are renewable, biodegradable, and environmentally friendly (Nemet et al., 2010).

Edible films are defined as thin layers of biological materials that are safe from a health point of view, approved by international food organizations and conform to the specifications related to food products. They are eaten with food (Senturk et al., 2018). Gelatin is widely used in various industrial applications due to its functional properties. In recent years, and due to consumer awareness research has directed investment in the field of preparing edible films such as coating cheese and meat. It has been used alone or with a mixture of fatty substances, proteins, and gums to form edible wrappers which are distinguished for improving food characteristics and extending their shelf life as a result of their ability to carry antioxidants and microbial inhibitors (Ramos et al., 2016).

CuO nanoparticles have gained great interest due to their unique chemical, thermal, biological, optical, and electrical properties. The antibacterial properties of CuoNPs have made them a valid choice for therapeutic uses (Qamar et al., 2020). In general, metal oxide nanoparticles produce reactive oxygen radicals (ROS) which are responsible for their antibacterial activity (Amiri et al., 2017). Selenium nanoparticles are also a good choice to be used as antimicrobial growth agents due to their unique chemical and morphological properties. It has been used as a preservative by coating metal cans used to preserve food. Its effectiveness has also been tested against many pathogenic microorganisms that contaminate humans and their food (Rajan et al., 2019).

The current study aimed to know the effectiveness of edible coating of gelatin loaded with copper oxide and selenium nanoparticles on some properties of soft cheese.

Material & Method

The method of making soft cheese

The cheese was manufactured according to the method (Fox et al., 2017) as follows:

The milk obtained from one of the milk processors in the city of Mosul. It was pasteurized at a temperature of 72 C for 15 seconds .After cooling to 35–40 C, rennet was added to it at a rate of 1 g per 25 liters of milk supplied by Meito Sangyo CO., LTD. The Japanese, where a solution of rennet powder was prepared in advance according to the instructions on the package The milk was mixed for 3 minutes and left to stagnate for 45 minutes until the cheese stage obtained . Then the curd was cut to get rid of the whey. Salt was added at a rate of 1%, after which the coagulation was placed in metal molds which were lined with a piece of wet cloth to get rid of the largest amount of whey and to prevent the leakage of some pieces of coagulation. The weights were placed for 1-2 hours, then the they were lifted. The molds were moved to the refrigerator until Cutting and packaging for direct microbial and chemical tests.

prepare gelatin films loaded with nanoparticles of copper oxide and selenium

Gelatin film were prepared according to the method of Chowdhury and Das (2012).

Packaging of cheese samples

Cheese samples were cut in a rectangular shape with a weight of 10 g per sample to ensure that the envelopes completely contain the samples. They were covered with gelatin films prepared in advance and loaded with nanoparticles of copper oxide and selenium. Then they were stored in the refrigerator until testing was carried out and according to the suggested storage periods.

The properties of soft cheese coated with gelatin films were tested

Chemical tests of soft cheese

Estimate the percentage of moisture:

The moisture content was estimated according to the method of Ling (2008) with a weight of 2 g of cheese, then it was dried using an electric oven at a temperature of 105 °C until the weight stabilized The percentage of moisture was calculated as in the following equation:

$$\%\text{Moisture} = \frac{\text{Weigh the sample before drying- Weigh the sample after drying}}{\text{Weigh the sample}} \times 100$$

Estimation of protein percentage: Protein was estimated according to the method described by Hool et al., (2004).

Fat percentage estimation: Kerber method reported by Min and Ellefson (2010) was used.

Estimation of Ash Percentage: Ashes were estimated by the direct burning method described in A.O.A.C (2004).

Measurement of pH: 10 gm of soft cheese was weighed and mixed well in a ceramic mortar with 10 ml of distilled water. A pH-meter was used to estimate the pH value of the sample (Ling., 2008).

Statistical analysis: The data were analyzed statistically according to the SAS statistical analysis program by testing the significant differences between the averages using the Complete Randomized Design (CRD) system, and the comparison between the averages was done by Dunkin's multiple test at the level of probability 0.05.(Steel and Torrie, 1980).

Results and discussion

Characteristics of soft cheese coated with gelatinous films

The chemical composition of soft cheese

Moisture percentage

Table (1) shows the results of moisture content in uncoated soft cheese samples that are coated with gelatin film only and gelatin films added to copper oxide nanoparticles and selenium nanoparticles prepared from the plant extract of *Hibiscus sabdariffa* L. and chamomile flowers, probiotics *Lactobacillus acidophilus* and *Lactobacillus plantarum*, and the mixed booster stored for 20 days at a temperature of 5±2 °C. The results showed that the moisture on the first day and for all treatments T1, T2, T3, T4, T5, T6, T7, T6, T7, T8, T8, T9, T8, T9, T10, T11, and T12 were 60.34, 60.72, 60.90, 60.69, 60.4, 60.75, 60.80, 60.59, 60.94, 60.55, 60.55 and 60.53 respectively. Where the moisture percentage for all samples was compatible with the Iraqi standard specification for soft cheese (the moisture percentage should not be less than 50%),. A decrease was observed in the moisture percentage on day 10 for all treatments, where the moisture percentage of the comparison sample without a membrane was 56.74%, while the moisture percentage of the coated cheese sample was only 58.6%. When compared with the treatments at the end of the storage period, it was found that the lowest moisture percentage was 51.43% for the cheese sample without wrapping T1. While the best treatment in its ability to preserve moisture was the T8 treatment that was coated with gelatin of the addition of nanoparticles of copper oxide. it was observed that the treatments coated with gelatin with the selenium nanoparticles and copper oxide addition were better in their ability to maintain the moisture content of cheese throughout the storage period compared to cheese without packaging. The results agreed with the findings of Ahmed (2020), who indicated that the moisture content of cheeses coated with gelatin films to which silver and zinc nanoparticles were added decreased slightly compared to the treatment of uncoated cheese. The results also agreed with what was stated by Al-Jubouri (2021), who showed the ability of the gelatinous membranes with the addition of nanoparticles to preserve the moisture content of the brick until the end of the storage period compared to the control sample.

Table 1.The effect of different treatments on the percentage of moisture in soft cheese coated with gelatin films and uncoated and stored for 20 days at a temperature (2 ± 5 °C).

Treatment	storage period		
	Day 1	Day10	Day20
T1	60.34 a	56.74 b-g	51.435 i
T2	60.72 a	58.6 a-d	55.230 d-h
T3	60.90 a	59.425 a-c	55.315 d-h
T4	60.69 a	59.689 ab	55.905 c-h
T5	60.4 a	58.72 a-d	54.55 e-i
T6	60.75 a	56.69 b-g	54.50 f-h
T7	60.80 a	57.70 b-f	55.34 d-h
T8	60.59 a	58.285 a-f	56.62 b-g
T9	60.94 a	57.60 e-i	53.62 g-i
T10	60.55 a	55.525 d-h	52.265 hi
T11	60.66 a	57.834 a-e	55.650 c-h
T12	60.53 a	55.825 c-h	53.260 g-i

* The numbers in the table are average for duplicates

* The different letters in one column indicate that there are significant differences at the level of 0.05%.

T1 = control sample T2 = cheese and membrane only T3 = membrane + Lacto.acidophlius SeNPs T4 = membrane + Lacto SeNPs. Plant. T5= membrane + Lacto-SeNPs. T6=Membrane +SeNPs prepared from the extract of *Hibiscus sabdariffa* T7=Membrane +SeNPs prepared from extract of chamomile T8=Membrane +CuoNPs Lacto. acidophilus T9=membrane + Lacto CuoNPs. Plant. T10=Cuo-Lacto.Mix+film T11=CuoNPs+film prepared from of *Hibiscus sabdariffa* extract T12=CuoNPs+film prepared from chamomile extract.

The percentage of protein

Table (2) shows the percentage of protein in uncoated soft cheese samples that were coated with gelatin films and gelatin films added to selenium nanoparticles (SeNPs) and copper oxide (CuoNPs) stored for 20 days at a temperature of 5 ± 2 °C. As the protein percentage for treatments T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, and T12 for the first day was at 18.30, 18.16, 18.2, 18.06, 18.3, 18.11, 18.29, 18.31, 18.26, 18.15, 18.20, and 18.10%, respectively. It was also observed that the protein percentages increased gradually with the progression of the storage period for all the above treatments. The protein percentage at the end of the storage period reached 19.20, 19.45, 19.49, 19.48, 19.35, 19.42, 19.22, 19.01, 19.18, 19.26, 19.26, and

19.30%, respectively. When comparing the treatments, it was found that the highest protein percentage was in the T4 treatment (19.49%), while the T8 treatment was 19.01%, and the lowest protein percentage . The results agreed with what was stated by Ahmed (2020), who found that the protein percentage of cheeses coated with gelatin films to which silver and zinc nanoparticles were added gradually increased at the end of the storage period. The reason is attributed to the loss in the moisture content of the cheese. Also, the results agreed with what al-Jubouri (2023) stated. He indicated that the percentage of protein in soft cheese samples that were coated with whey protein membranes and to which zinc nanoparticles, lactoferrin, and bacteriocins were added gradually increased, and the highest percentage was for the T4 treatment reaching 20.55%. The results also agreed with what Zheng et al. (2018) concluded about the existence of a discrepancy in the proportions of protein in the cheese treatments The reason for these differences was the moisture content values and the chemical composition of the antimicrobial substances added to the coatings, as well as the presence of proteolytic bacteria.

Table 2.the effect of different treatments on the percentage of protein in soft cheese coated with gelatin films and uncoated and stored for 20 days at a temperature (2 ± 5 °C).

Treatment	Storage period		
	Day1	Day10	Day20
T1	18.30 j	18.5 h-j	19.20 c-d
T2	18.16 j	18.68 f-i	19.45 a-c
T3	18.2 j	18.31 j	19.49 a
T4	18.06 j	18.25 j	19.48ab
T5	18.3 j	18.62 g-i	19.35 a-d
T6	18.11 j	18.69-i-f	19.42 a-b
T7	18.29 j	18.76 f-h	19.22 a-d
T8	18.31 j	18.9 e -f	19.01 de
T9	18.26 j	18.81 fg	19.18 cd
T10	18.15 j	18.87 fg	d 19.26 –a
T11	18.20 j	18.45 ij	19.26 d-a
T12	18.10 j	18.31 j	19.30 a-d

* The numbers in the table are average for duplicates
* The different letters in one column indicate that there are significant differences at the level of 0.05%.

T1 = control sample T2 = cheese and membrane only T3 = membrane + Lacto.acidophlius SeNPs T4 = membrane + Lacto SeNPs. Plant. T5= membrane + Lacto-SeNPs. T6=Membrane +SeNPs prepared from the extract of *Hibiscus sabdariffa* T7=Membrane +SeNPs prepared from extract of chamomile T8=Membrane +CuoNPs Lacto. acidophilus T9=membrane + Lacto CuoNPs. Plant. T10=Cuo-Lacto.Mix+film T11=CuoNPs+film prepared from of *Hibiscus sabdariffa* extract T12=CuoNPs+film prepared from chamomile extract.

Fat percentage

The results shown in Table (3) the percentage of fat for uncoated soft cheese that was coated with gelatin films and gelatin films with the addition of selenium nanoparticles (SeNPs) and copper oxide (CuoNPs) and stored for 20 days at a temperature of 5 ± 2 C. The readings show that the percentage of fat for all treatments for the first day was 16%, and this percentage continued to increase significantly until the end of the storage period. When the percentage of fat for treatments T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, and T12 reached 17, 19, 18, 18, 18. 19,19,19, 17, 17, 17, 17%, respectively. The highest value of fat for the treatments T2, T6, T7, and T8 was 19% at the end of the storage period. This increase in the percentage of fat with the progression of the storage period is attributed to the decrease of moisture content; as the percentage of moisture decreases, it leads to an increase in the percentage of other components of the cheese. These results are consistent with what Ahmed (2020) concluded. He found that the percentage of fat in soft cheese coated with gelatin films to which silver and zinc nanoparticles were added increased at the end of the storage period of the cheese. Also, the results agreed with what was stated by Al-Jubouri (2023. The percentage of fat in soft cheese coated with whey protein membranes to which zinc nanoparticles and lactoferrin were added increased at the end of the storage period. The highest percentage of fat reached 19.13%. The increase in fat content with the progression of the storage period may be attributed to the decrease in moisture content (Zheng et al., 2018).

Table 3.the effect of different treatments on the percentage of fat in soft cheese coated with gelatin films and uncoated and stored for 20 days at a temperature (2 ± 5 °C).

Treatment	Storage period		
	Day 1	Day 10	Day20
T1	16 a	17 a	17 a
T2	16 a	17 a	19 a
T3	16 a	18 a	18 a
T4	16 a	18 a	18 a
T5	16 a	18 a	18 a
T6	16 a	19 a	19 a
T7	16 a	19 a	19 a
T8	16 a	18 a	19 a
T9	16 a	17 a	17 a
T10	16 a	17 a	17 a
T11	16 a	17 a	17 a
T12	16 a	16 a	17 a

* The numbers in the table are average for duplicates
* The different letters in one column indicate that there are significant differences at the level of 0.05%.

T1 = control sample T2 = cheese and membrane only T3 = membrane + Lacto.acidophlius SeNPs T4 = membrane + Lacto SeNPs. Plant. T5= membrane + Lacto-SeNPs. T6=Membrane +SeNPs prepared from the extract of *Hibiscus sabdariffa* T7=Membrane +SeNPs prepared from extract of chamomile T8=Membrane +CuoNPs Lacto. acidophilus T9=membrane + Lacto CuoNPs. Plant.

T10=Cuo-Lacto.Mix+film T11=CuoNPs+film prepared from of *Hibiscus sabdariffa* extract T12=CuoNPs+film prepared from chamomile extract.

Ash percentage

Table (4) shows the results of the percentage of ash in soft cheese uncoated and coated with gelatin films and gelatin films with the addition of nanoparticles of selenium SeNPs and copper oxide CuoNPs prepared from plant extracts of *Hibiscus sabdariffa* L., chamomile, and probiotics and stored for 20 days at a temperature of 5 ± 2 °C, where the ash percentage on the first day was 0.90, 0.92,0.91,0.94,0.96, 0.93, 0.92, 0.97, 0.95, 0.94, 0.92, and 0.96% for transactions T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, and T12, respectively. The results also showed that there were significant differences on day 10 between all treatments. A gradual increase was observed in the percentage of ash in soft cheese and for all treatments at the end of the storage period. The significant differences of all treatments T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, and T12, as the highest percentage of ash reached 1.78, 1.665, 1.64, 1.68, 1.67, 1.74, 1.71, 1.395, 1.50, 1.66, 1.305, and 1.836%, respectively. The highest value of ash was 1.836% for treatment T12, while the lowest percentage was 1.305% for treatment T11. These results were consistent with what Ahmed (2020) reported about the increase in the percentage of ash in soft cheese with the progression of the storage period covered with gelatin to which silver nanoparticles and nanoparticles of zinc were added. The highest percentage of ash at the end of the storage period reached 1.84%, while the percentage of ash in the controled sample was 1.70%. Dimitrellou et al. (2015) indicated that the reason for the increase in ash content during the storage period was due to the decrease in humidity in the treatments. The results agreed with what Al-Jubouri (2023) found, who found that the ash percentage increased with the progression of the storage period for soft cheese coated with whey protein films, to which nano-zinc and lactoferrin were added, as the highest ash percentage reached 1.90% while the lowest ash percentage was 1.78%.

Table 4. Effect of different treatments on the percentage of ash in soft cheese coated with gelatin films and uncoated and stored for 20 days at a temperature (5±2°C).

Treatment	Storage period		
	Day1	Day10	Day20
T1	0.90 o	1.375 k-m	1.78 ab
T2	0.92 o	1.560 f-h	1.665 c-e
T3	0.91 o	1.415 i-k	1.64 c-f
T4	0.94 o	1.48 h-j	1.68 cd
T5	0.96 o	1.49 j-h	1.67 c-e
T6	0.93 o	1.59 d-g	1.74 bc
T7	0.92 o	1.57 e--h	1.71 bc
T8	0.97 o	1.285 mn	1.395 j-l
T9	0.95 o	1.35 k-m	1.50 g-i
T10	0.94 o	1.215 n	1.66 c-e
T11	0.92 o	1.23 n	1.305 l-n
T12	0.96 o	1.655 c-f	1.836 a

* The numbers in the table are average for duplicates
* The different letters in one column indicate that there are significant differences at the level of 0.05%.

T1 = control sample T2 = cheese and membrane only T3 = membrane + Lacto.acidophlius SeNPs T4 = membrane + Lacto SeNPs. Plant. T5= membrane + Lacto-SeNPs. T6=Membrane +SeNPs prepared from the extract of *Hibiscus sabdariffa* T7=Membrane +SeNPs prepared from extract of chamomile T8=Membrane +CuoNPs Lacto. acidophilus T9=membrane + Lacto CuoNPs. Plant. T10=Cuo-Lacto.Mix+film T11=CuoNPs+film prepared from of *Hibiscus sabdariffa* extract T12=CuoNPs+film prepared from chamomile extract.

pH values of coated soft cheese samples

Table (5) shows the pH values of soft cheese samples coated with gelatin. Gelatin films added to selenium particles and copper nanoparticlescompared to uncoated cheese sample. The pH value on the first day of storage was 6.80, 6.82, 6.82, 6.81, 6.78, 6.80, 6.79, 6.78, 6.79, 6.79, 6.80, and 6.82% for transactions T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, and T12, respectively. While the pH values for the 10th day ranged between 6.57 and 6.65 for the treatments coated with gelatin films to which selenium nanoparticles and copper oxide were added being compared to the treatments T1 and T2. The two control samples without coating and coated with gelatin only amounted to 6.60 and 6.7, respectively. These results agree with the standard. The Iraqi standard for the year 1988, as the Iraqi standard specification for soft cheese states that the pH value of soft cheese ranges between (6.4 ± 0.2). With the continuation of the storage period, a decrease was observed in the pH values with significant differences between all treatments T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, and T12, as the pH values were 5.4, 6.3, 6.2, 6.3, 6.1, 6.2, 6.2, 6.1, 6.2, 5.9, 6.1, and 6.2, respectively. These results agreed with what was reached by Al-Jubouri (2023) for soft cheese coated with membranes of evil proteins to which zinc nanoparticles and lactoferrin were added The pH value of the comparison sample per day The first was 6.76 compared to the sample of cheese covered with a film to which nano-zinc was added, as the pH value was 6.77, while the pH values of the comparison sample and the sample coated with whey protein membranes to which zinc nanoparticles were added at the end of the storage period were 6.06 and 6.25, respectively. The reason for the decrease in the pH value in the soft cheese samples is attributed to the process of fermentation of the remaining lactose sugar in the cheese samples after separating the whey affected by the microbial content and the moisture content that affects the activity of microorganisms (Fathollahi et al., 2010). The results agreed with what was found by Al-Jubouri (2021) He indicated that there was a significant decrease in labneh samples coated with gelatin films to which chitosan particles and titanium nanoparticles were added at the end of the storage period, compared to the two control samples without coating and coated with gelatin only.

Table 5. The effect of different treatments on the pH values of soft cheese coated with gelatin films and uncoated and stored for 20 days at a temperature (2±5°C).

Treatment	Storage period		
	Day1	Day10	Day20
T1	6.80 a	6.60 ab	5.4 e
T2	6.82 a	6.7 a	6.3 bc
T3	6.82 a	6.6 ab	6.2 bc
T4	6.81 a	6.65 a	6.3 bd
T5	6.78 a	6.69 a	6.1 cd
T6	6.80 a	6.65 a	6.2 cd
T7	6.79 a	6.62 ab	6.2 cd
T8	6.78 a	6.61 ab	6.1 cd
T9	6.79 a	6.59 ab	6.2 cd
T10	6.79 a	6.58 ab	5.9 d
T11	6.80 a	6.57 ab	6.1 cd
T12	6.82 a	6.57 ab	6.2 cd

* The numbers in the table are average for duplicates
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T1 = control sample T2 = cheese and membrane only T3 = membrane + Lacto.acidophlius SeNPs T4 = membrane + Lacto SeNPs. Plant. T5= membrane + Lacto-SeNPs. T6=Membrane +SeNPs prepared from the extract of *Hibiscus sabdariffa* T7=Membrane +SeNPs prepared from extract of chamomile T8=Membrane +CuoNPs Lacto. acidophilus T9=membrane + Lacto CuoNPs. Plant. T10=Cuo-Lacto.Mix+film T11=CuoNPs+film prepared from of *Hibiscus sabdariffa* extract T12=CuoNPs+film prepared from chamomile extract.

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Competing Interests:
The authors declare no conflicts of interest**.**

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